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Thrombosis and Restenosis after Peripheral Angioplasty: Does Acute ^{111}Indium-platelet Accumulation Predict Angioplasty Outcome?

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Objectives: *Use of platelet deposition to predict failure following angioplasty.*
Design: *Prospective study of angioplasty patients in a 9 months surveillance period.*
Materials: *Thirty-eight successful angioplasty patients.*
Methods: *Autologous ^{111}indium-labelled platelets were re-injected immediately after angioplasty. Gamma camera and probe measures of radioactivity were obtained daily for 2–4 days and compared to a reference site to obtain a radioactivity ratio. Patient follow up was with duplex and arteriography from day 0 to 9 months or at angioplasty failure.*
Results: *Thirty-one patients remained asymptomatic; two developed acute occlusion and five developed restenosis. Platelet accumulation (increased mean radioactivity ratio) occurred at all angioplasty sites and was significantly higher after acute occlusion (camera: 2.93 and probe: 1.93) compared to asymptomatic patients (camera: 1.25 and probe: 1.15) and restenotic patients (camera: 1.31 and probe: 1.23). Radioactivity ratio was not different in patients who later developed restenosis.*
Conclusion: *^{111}Indium platelet radioactivity effectively detected acute angioplasty reocclusions, but was unable to predict subsequent angioplasty restenosis.*

Key Words: ^{111}Indium-platelets; Thrombosis; Angioplasty; Restenosis.

Introduction

Platelets may play a significant role in the development of experimental restenosis both directly, by thrombosis and indirectly, through the production of mediators which stimulate the development of fibrocellular intimal hyperplasia.[1] Platelet derived growth factor, one such mediator is chemotactic for medial smooth muscle cells; it also interacts with insulin derived growth factor and thrombin to stimulate smooth muscle cell proliferation during the formation of intimal hyperplasia.[2] Such smooth muscle cell migration and proliferation underlie the changes at the sites of anastomotic stenosis and angioplasty restenosis. ^{111}Indium-labelled platelets permit clinical detection of thrombus and give an estimation of platelet dynamics. This radioactive tracer has demonstrated thrombus formation in native arteries and thrombosis and restenosis at arterial anastomotic sites.[3,4]

The aim of this prospective clinical study was to assess the significance of acute platelet accumulation at angioplasty sites on angioplasty outcome by determining the influence of acute radioactivity detection from autologous ^{111}indium-labelled platelets.

Materials and Methods

Autologous platelets were harvested from 50 ml of venous blood and labelled by a standard technique and used to assess platelet accumulation after successful femoropopliteal artery angioplasty.[5] ^{111}Indium-labelled platelets were injected after completion of the angioplasty procedure, at the time of manual compression at the puncture site. Angioplasty surveillance was performed with ankle-brachial Doppler pressure indices (ABPI) and colour duplex imaging at 1, 2 and 3 days and 1, 3, 6 and 9 months after angioplasty. The daily tests were done immediately before each radioactivity assessment. Patients were also assessed

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by intravenous digital subtraction arteriography (IV-DSA) at 6 months or, if sooner, at the time of angioplasty failure.

Activity was measured at the angioplasty site and at a similar position (of known arterial patency) in the opposite limb using a single probe and a medium field of view gamma camera. Counts were obtained at 1–3 h (day 0), 18–21 h (day 1), 40–44 h (day 2) and 64–68 h (day 3) after treatment as follows: For the probe studies, three measurements of radioactive counts were made over a 10 s period by holding the probe perpendicular to the skin surface and centred on permanent ink skin marks (placed at the time of angioplasty) over the treated site and then over the reference site. For the gamma camera, images were centred on the ink marks and the treated site was labelled with a cobalt-57 point source. The gamma image included both lower limbs from the symphysis pubis to the knees for superficial femoral artery lesions, and from the umbilicus to the mid-femoral region for iliac lesions. The cobalt mark was overlapped with the gamma camera images to facilitate identification of the exact treatment site. The gamma images alone (without the cobalt source) were then analysed using regions of interest (ROI) as follows: a region of interest was drawn over the angioplasty site, and radioactivity counts were measured within the drawn area. The same region of interest was used to measure the activity from the reference artery. In order to eliminate the differences in background radioactivities in different patients and at different sites the ratio of counts (treated site vs. reference site) was calculated and used for comparison. Because the area of the angioplasty site was variable three variably sized boxes were drawn over the treated region in each patient and at each time point. Thus three consecutive measurements were made from the same image by the same observer using different ROI. The mean of the three ratios was calculated and called the radioactivity ratio (RR). Similar calculations were made for the probe counts. Thus, two RRs were obtained at each assessment time, one for the gamma camera and one for the single probe measurements. Radioactivity measurements were stopped when an occlusion was objectively demonstrated.

Percent diameter stenosis (PDS) was measured from angiograms and duplex images as the ratio of the diameter reduction at the angioplasty site to the diameter of the 'normal' artery lumen (e.g. no lumen = 100% diameter stenosis). Duplex diameters were assessed by measuring the colour-filled lumen on a longitudinal image of the artery. The point of maximum systolic velocity at the angioplasty site was used and compared to a normal artery segment and at three different probe positions. This study was approved by the Joint UCL/UCH Committee on the Ethics of Human Research, Middlesex Hospital Clinical Investigations Panel. Informed written consent was obtained from all patients.

Results

Angioplasty procedures were performed in 38 patients with an age range from 42 to 80 years. There were 24 men and 14 women. All patients had life-style limiting lower limb claudication and angiographically demonstrated stenoses (30) or occlusions (8). Patients were given prophylactic heparin (2000 units) and 75 mg of aspirin was given daily after angioplasty. Two patients reoccluded their angioplasty site within 24 h (1 dilated femoropopliteal stenosis and 1 dilated femoropopliteal occlusion). Five patients developed clinical angioplasty failure signalled by recurrence of symptoms and confirmed by duplex and angiography. These all required re-intervention. Thirty-one patients remained asymptomatic during the 9 month follow-up period.

The labelling efficiency was in the order of 80% and the average radioactivity administered to patients was 16.5 MBq (range 14.1–21.6 MBq). There was progressive reduction in the absolute daily radioactivity counts with time. Radioactivity measurements were highest at the initial measurement and there was progressive reduction in absolute counts over subsequent days. All patients showed higher counts on the treated side than the normal side. The mean RRs with the probe were 1.15, 1.23 and 1.93 for asymptomatic patients, late angioplasty failures (restenosis) and acute reocclusions respectively. The camera values were 1.25, 1.31, 2.93 for the same three groups. The increased activity after acute occlusion were significantly different from those seen in asymptomatic patients and angioplasty failures (significant at 99% ANOVA) (Fig. 2). The values were slightly higher for patients who developed restenosis than for asymptomatic patients but this did not reach statistical significance.

The influence of other patient factors on the RR were also assessed and the data is summarised here: clinical factors (pre-angioplasty aspirin medication and smoking status), differences in the cannulation procedure (guidewire, laser fibre-guided, laser-guided) and angiographic factors (degree of arterial trauma estimated from dissections and flaps, number of stenoses treated and the length of lesion) showed no significant influence on RR for either camera or probe counts. RR showed a difference on camera count when angioplasty was performed for stenoses rather than occlusions.

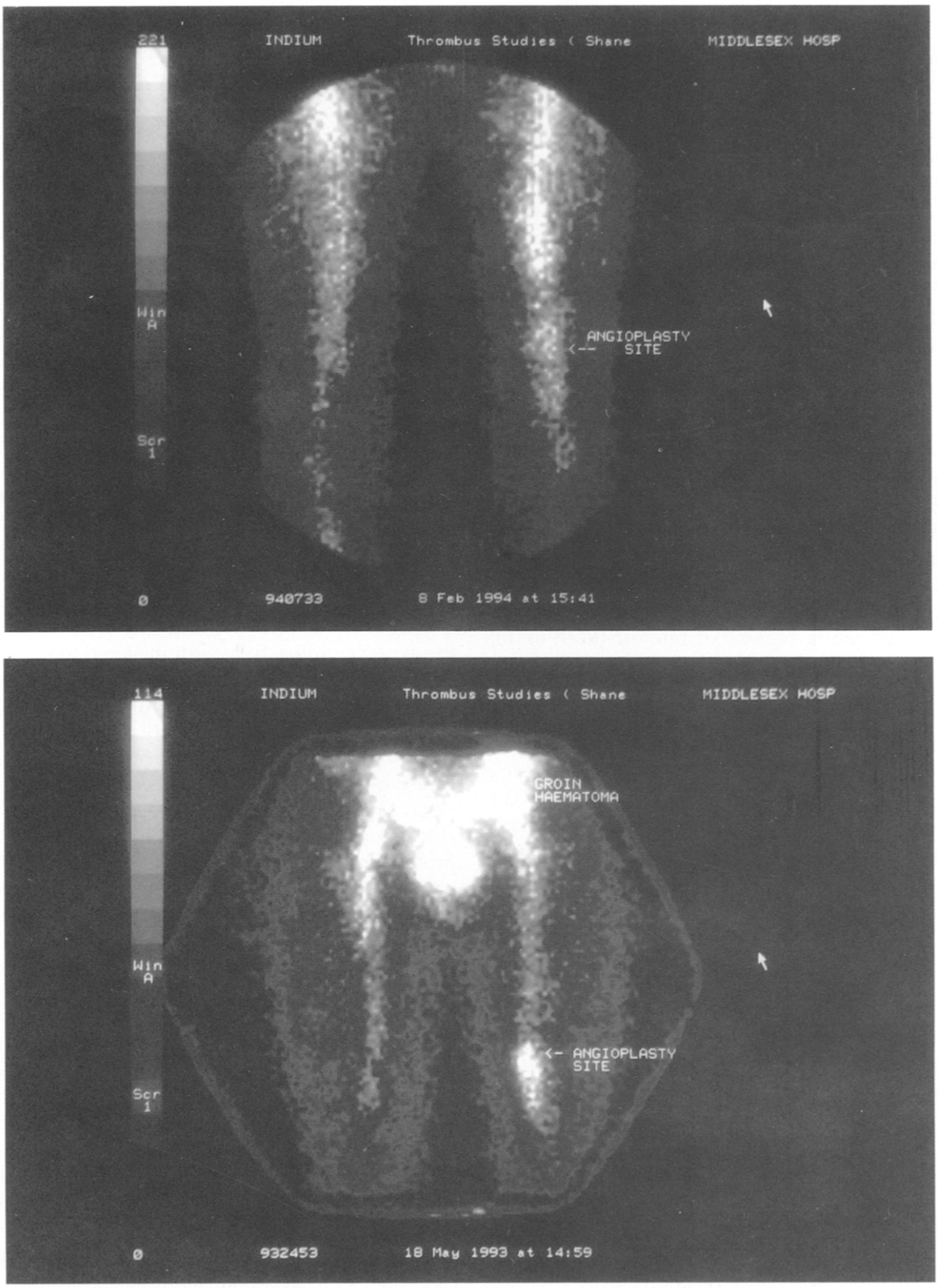

Fig. 1. (a) ^{111}Indium image obtained 1 day after left superficial femoral artery angioplasty in a patient with a patent angioplasty site. (b) ^{111}Indium image obtained 1 day after angioplasty in a patient after left superficial femoral artery angioplasty, who has developed acute occlusion at the angioplasty site. Both images show increased radioactivity at the angioplasty treated side (arrow) compared to the untreated side. There is greater radioactivity in the presence of acute occlusion.

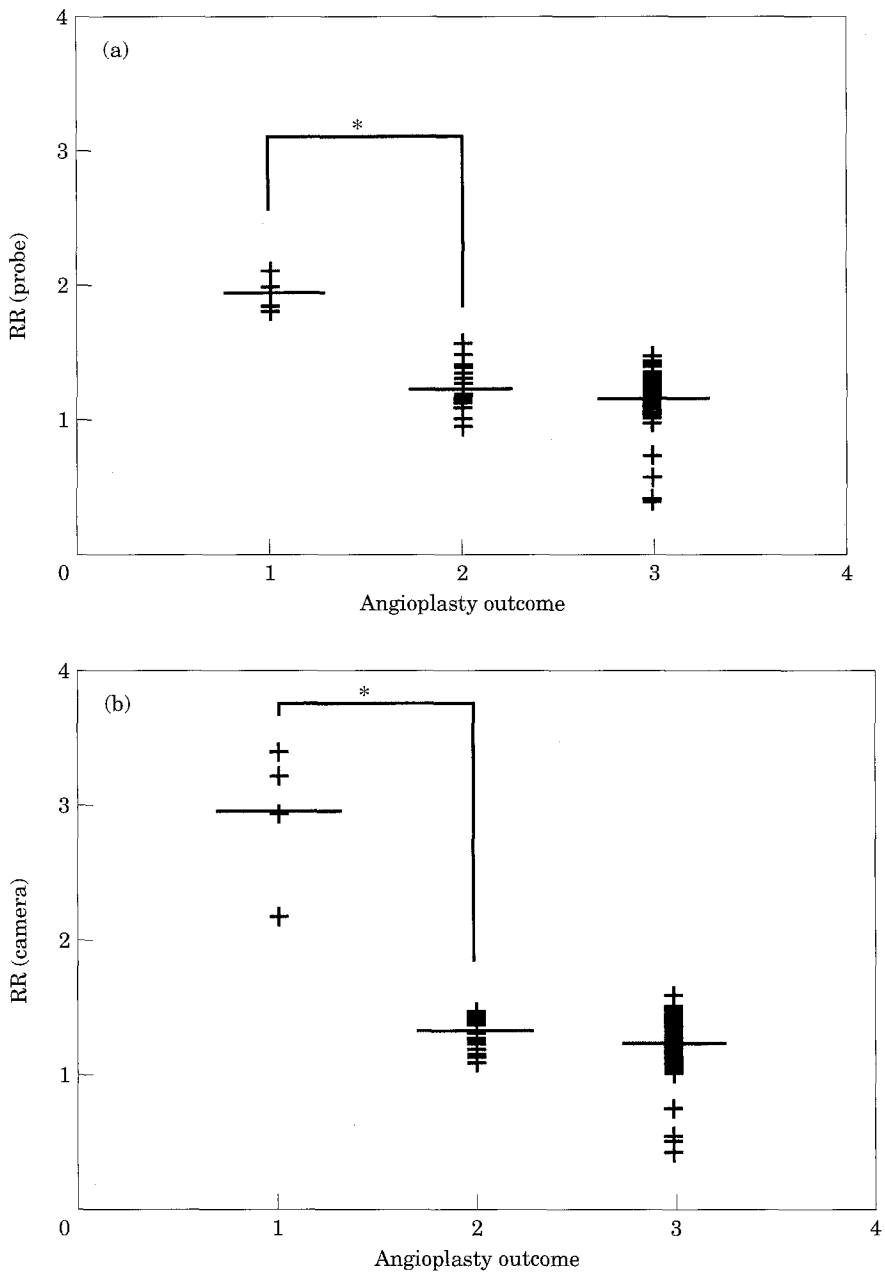

Fig. 2. Radioactivity ratio (RR) and angioplasty outcome. (a) Graph showing radioactivity ratios (RR) measured by probe as a function of angioplasty outcome. (b) Graph showing radioactivity ratios (RR) measured by camera as a function of angioplasty outcome. Outcome groups are represented as follows: 1 – reocclusion within 24 h, 2 – angioplasty failures (who required re-intervention between 1 day and 9 months, and 3 – patent arteries at 9 months. *Significant at 99% ANOVA.

Discussion

^{111}Indium-platelet labelling is an established method for assessing platelet deposition *in vivo*. We have studied the effects of acute platelet deposition on angioplasty outcome by detecting ^{111}indium-platelet deposition and showed that it did not predict angioplasty restenosis and this may have been due in part to bleeding into the arterial wall. A marked rise in radioactivity counts in our patients always coincided with acute angioplasty occlusion. These counts differed significantly from those patients who did not show acute occlusion. Radioactivity ratios were generally higher for camera measurements, where arterial site specificity was assured, than for probe measurements. Excluding the acute occlusions, rise in radioactivity counts were not different in patients whose angioplasty sites remained patent from those who developed symptoms because of restenosis, although there was a trend of slightly higher values in the latter. A larger study with greater statistical

power might reveal this difference to be significant, however, it is unlikely that such a difference would have any value in predicting outcome in individual patients. This was in agreement with experimental and clinical reports which showed that reduction of platelet/vessel wall interactions did not influence restenosis.[4,5] Our findings, however, contradict previous studies with small patients numbers which reported localised increase in radioactivity after angioplasty to be predictive of restenosis.[8–10]

The role of platelets in restenosis is complex and may not be easily explained by detection of platelet deposition at angioplasty sites. Angioplasty causes endothelial denudation which stimulates platelet adhesion and aggregation to the underlying subendothelial surface. Active chemotactic and mitogenic agents released during this platelet reaction stimulates smooth muscle cell interactions involved in the formation of intimal hyperplasia.[11] Despite the role of platelets in these smooth muscle cell interactions, antiplatelet drugs such as aspirin and dypiridamol, platelet function inhibitors such as ticlopidine, and platelet anti-aggregates have all failed to reduce clinical restenosis.[12–14] In addition, experimental studies of antiplatelet antibodies which have reduced thrombus formation have not influenced experimental restenosis. Monoclonal antiplatelet antibody induced thrombocytopenia caused marked reduction but did not completely abolish experimental intimal hyperplasia or clinical restenosis.[15,16] Thus the role of adherent platelets at angioplasty sites in the subsequent formation of intimal hyperplasia remains unclear.

The reasons why acute platelet detection does not signal later angioplasty restenosis are not understood. We speculate that the effective production of platelet stimulatory factors may be achieved by the initial layer of adhering platelets, and further platelet aggregation may be superfluous to the subsequent smooth muscle cell interactions. Also, many of the interacting platelet factors are produced by other cells such as endothelial cells, smooth muscle cell and monocyte-macrophages within the injured arterial wall so that platelets may not be essential for restenosis.[17]

In this report ^{111}indium-platelets detected acute occlusions although such failures occurred in only two of our primary angioplasty successes. This is the subject of another report.[18] ^{111}Indium-platelet deposition failed to indicate the treatment method used at angioplasty. This probably reflects the fact that balloon dilatation, the major traumatic event, is common to all the procedures. These results contrast with Ram Mohan *et al.* and others who have reported an increased incidence of thrombosis for laser assisted balloon angioplasty.[9]

It is a criticism of the present report that the number of acute and chronic angioplasty failures are small and our comments should be viewed in this light. In this study of modest patient numbers we have found no evidence to support a useful role for ^{111}indium-platelets and radioactivity counts in predicting angioplasty restenosis. Thus it could offer no help in predicting patients for prophylactic therapy or for intensive angioplasty surveillance. Predictably, radioactivity counts detected all acute thrombotic occlusions. However, platelet studies are expensive and are unlikely to have a useful clinical role because such occlusions are readily detected by duplex scanning. The number of patients who developed acute occlusion in this study were too small to allow meaningful conclusions on aetiological factors to be made. The process of ^{111}indium-platelet labelling and radioactivity detection is expensive and labour intensive and this method must now be considered to have a doubtful role in the study of angioplasty restenosis.

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